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Inflammation Research

Is there a role of taurine bromamine in inflammation? Interactive effects with nitrite and hydrogen peroxide

J. Marcinkiewicz[1], M. Mak[1], M. Bobek[1], R. Biedroń, A. Białecka[2], M. Koprowski[1], E. Kontny[3] and W. Maśliński[3]

[1] Department of Immunology, Jagiellonian University Medical College, 18 Czysta St., 31-121 Craców, Poland, Fax: ++4812 633 94 31, e-mail: mmmarcin@cyf-kr.edu.pl

[2] Center of Microbiological Research and Autovaccines Ltd., Cracow, Poland,

[3] Department of Pathophysiology and Immunology, Institute of Rheumatology,Warsaw, Poland

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Abstract. *Objective and Design:* The myeloperoxidase system of neutrophils generates chlorinating and brominating oxidants in vivo. The major haloamines of the system are taurine chloramine (TauCl) and taurine bromamine (TauBr). It has been demonstrated in vitro that TauCl exerts both anti-inflammatory and anti-bacterial properties. Much less is known about TauBr. The present study was conducted to compare bactericidal and immunoregulatory capacity of TauBr with that of the major chlorinating oxidants: HOCl and TauCl. Moreover, the effect of nitrites and H_2O_2 on TauBr activity was investigated.
Materials: TauBr was prepared by reaction of HOBr with taurine. The reaction was monitored by UV absorption spectra.
Methods: Bactericidal activity of TauBr, TauCl and HOCl was tested by incubation of *E. coli* with the compounds and determined by the pour-plate method. To test the anti-inflammatory activity the compounds were incubated with LPS- and IFN-γ stimulated murine peritoneal macrophages. The production of following mediators was measured: nitrites by Griess reaction; TNF-α, IL-6, IL-10, IL-12p40 using capture ELISA. In some experiments the compounds were incubated with either nitrites or H_2O_2.
Results: In our experimental set-up TauBr and HOCl exerted strong bactericidal effects on *E. coli* (MBC = 110 µM and 8 µM, respectively), while TauCl (< 1000 µM) did not kill test bacteria. However, both, TauBr and TauCl, at non-cytotoxic concentrations (< 300 µM) inhibited the cytokine and nitric oxide production by macrophages. H_2O_2 completely abolished the biological activities of TauBr but not those of TauCl. Nitrites did not affect any activity of TauBr or TauCl while they diminished the $HOCl^-$ mediated bacterial killing.
Conclusion: TauBr, despite very low concentration of Br^- in body fluids, may support TauCl and HOCl in the regulation of inflammatory response and in killing of bacteria by neutrophils. However, TauBr activity in vivo will depend on the presence of H_2O_2 and possible other mediators of inflammation which can compete with target molecules for TauBr.

Key words: Inflammation – Neutrophils – Macrophages – MPO-halide system – Taurine bromamine – Taurine chloramine – Hydrogen peroxide – Bactericidal activity – Cytokine secretion

Correspondence to: J. Marcinkiewicz

Introduction

Activated neutrophils and eosinophils generate a variety of reactive oxygen intermediates (ROI) including hypohalous acids (HOCl, HOBr), the products of peroxidase-halide system [1–4]. Myeloperoxidase (MPO), the heme enzyme synthesized/secreted by neutrophils and monocytes, uses H_2O_2 and Cl^- to produce hypochlorous acid (HOCl), its major initial product [5, 6].

$$Cl^- + H_2O_2 + H^+ \rightarrow HOCl + H_2O$$

HOCl is an unstable, highly reactive oxidant with strong bactericidal activity. MPO-halide system generates also secondary oxidants such as mono-chloramines, di-chloramines and amino acid-derived aldehydes [7, 8]. As taurine is the most abundant free amino acid in leukocyte cytosol [9], the major chloramine generated by activated neutrophils is taurine chloramine (N-chlorotaurine, TauCl) [8]. TauCl is less toxic than HOCl, long lived oxidant with well documented in vitro microbicidal activity [10–12]. Concerning biological functions, it is assumed that the formation of TauCl protects body cells from damage by HOCl [8, 13]. Moreover, data from several laboratories demonstrate that TauCl is a powerful regulator of inflammation [14–17]. TauCl exerts anti-inflammatory properties by suppressing the production of such mediators as nitric oxide, PGE_2, TNF-α, IL-6, IL-8, IL-12 and chemokines in both rodent and human leukocytes [14–18]. Studies investigating the mechanisms of action of

TauCl have shown that it inhibits the activation of NF-kB, a potent signal transducer for inflammatory cytokines, by oxidation of IkB-α at Met^{45} [19].

Eosinophil peroxidase (EPO), a heme protein structurally related to MPO, is released by activated eosinophils, which helps to kill invading parasites [4]. At plasma concentrations of halide (100 mM chloride; 20–100 µM bromide; < 1 µM iodide) eosinophil peroxidase preferentially oxidizes bromide (Br^-) to produce hypobromous acid, HOBr [4].

$$Br^- + H_2O_2 + H^+ \rightarrow HOBr + H_2O$$

Only recently it has been published that HOBr may be also generated by the myeloperoxidase system of neutrophils [4, 7].

$$HOCl + Br^- \rightarrow HOBr + Cl^-$$

HOBr is a weaker oxidant than HOCl, but exerts even stronger microbicidal and cytotoxic activity [3, 4, 21, 22]. At physiological pH, both HOCl and HOBr react readily with amines to form haloamines (chloramines, bromamines) and with the unsaturated bonds of fatty acids to form halohydrins [7, 10, 23]. In studies with isolated leukocytes, evidence for the formation of brominating agents was obtained, but long lived bromamines were not detected in the medium. Reduction of $HOBr^-$ and bromamines by H_2O_2 may account for these results [20]. Nevertheless HOBr reacts with amino acids to form bromoamines, and Taurine bromamine (N-bromotaurine, TauBr) is considered to be the major product of these reactions [3, 4]. In contrast to large body of evidence demonstrating the role of TauCl in inflammation [13–19, 24], much less is known about biological properties of TauBr [25, 26].

Activated eosinophils as well as neutrophils can simultaneously generate a variety of reactive oxygen species (ROS) and nitric oxide (NO) [1, 27]. It has been demonstrated that leukocyte derived oxidants such as nitric oxide (NO/NO_2^-) and hydrogen peroxide (H_2O_2) differentially react with HOCl, HOBr and their derivatives [28, 29]. For example, HOCl, HOBr and bromamines are reduced by H_2O_2 while chloramines are resistant [4, 20].

$$HOBr + H_2O_2 \rightarrow H_2O + H^+ + Br^- + O_2$$

NO and its more stable metabolites, nitrite (NO_2^-) and nitrate (NO_3^-) may also react with hypohalous acids to form novel biologically active species [30]. We have previously shown that nitrite, the product of NO degradation, inhibits bactericidal activity of HOCl [24]. On the contrary, nitric oxide potentiates hydrogen peroxide-induced killing of *Escherichia coli*, as described by others [31]. Thus, the interactions between ROS, NO and MPO-halide system products may results in both the enhancement and the inhibition of neutrophil bactericidal – cytotoxic activities determining the balance between immune defence and host injury.

The bactericidal and immunoregulatory activities of TauBr in the presence of ROS have never been investigated systemically.

The present study was conducted to evaluate some biological functions of TauBr. We have compared bactericidal, cytotoxic and anti-inflammatory potency of TauBr with that of TauCl and/or HOCl, the major products of MPO-halide system. Moreover, the effect of nitrite (NO_2^-) and hydrogen peroxide (H_2O_2) on the activity of TauBr has been tested.

Materials and methods

Preparation of taurine bromamine (TauBr) and taurine chloramine (TauCl)

TauCl was prepared by dropwise addition of 5 ml of 20 mM NaOCl (Aldrich, Steinham, Germany) solution in 0.05 M phosphate buffer (pH 7.4), with vigorous stirring, to 5 ml of 24 mM taurine (Tau) (Sigma, St. Louis MO). Each preparation of TauCl was monitored by UV absorption spectra (λ = 200 to 400 nm) to assure the authenticity of monochloramine (TauCl) (λ_{max} = 252 nm) and the absence of dichloramine ($TauCl_2$) (λ_{max} = 300 nm) and unreacted $HOCl/OCl^-$ (λ_{max} = 292 nm). The concentration of synthesized TauCl was determined using the molar extinction coefficient 429 M^{-1} cm^{-1} at A_{252} [23].

TauBr preparation: $HOBr/OBr^-$ was generated by mixing equimolar amounts of $HOCl^-$ and $NaBr^-$. Than, the product of this reaction was mixed with taurine. The mono-bromamine was obtained with a 10-fold excess of amine over the amount of $HOBr/OBr^-$. Each preparation of TauBr was monitored by UV absorption spectra (λ = 200 to 400 nm) to assure the authenticity of monobromamine (TauBr). Taurine bromamines (mono-, di-bromamine) have absorption spectra similar to those of chloramines, but shifted 36 nm towards longer wavelengths. TauBr and $TauBr_2$ give UV spectra with λ_{max} = 288 nm and λ_{max} = 241 nm, respectively [4].

The concentration of synthesized TauBr was determined using the molar extinction coefficient 415 M^{-1} cm^{-1} at A_{288}.

Stock solutions of TauCl and TauBr were kept at 4 °C for maximum 5 days before use.

Preparation of bacteria

E. coli (EC) (ATCC 25922) was grown in Trypticase soy broth (Difco, Detroid, MI) at 37 °C for 24 h. Bacteria were centrifuged at 1800 × g, washed twice in 0.9% NaCl, and diluted in saline to a concentration of 1×10^8 CFU/ml before use.

Bactericidal activity of TauBr, TauCl and HOCl

Basing on the kinetics studies of bacterial killing by neutrophils [12] and our preliminary experiments demonstrating time- and dose-dependent bactericidal and cytotoxic activity of TauBr, we have established experimental conditions for this study. In our experimental set-up, at concentrations up to 1 mM, neither H_2O_2, NO_2^- nor taurine affected viability of bacteria. Briefly, bacteria were suspended and further diluted in 0.2 M phosphate buffer (pH-7.4) to achieve a final concentration of 1×10^5 CFU/ml and then were incubated with different concentrations of the components (1 – 1000 µM). Immediately after the incubation (30 min.), aliquots were removed and the viable cell count was determined by the pour-plate method [28]. Control samples (bacteria diluted in the buffers only), were treated the same way. Bactericidal activity of TauBr, TauCl and HOCl are expressed either as a log CFU/ml of bacteria survived at the indicated concentration of the component or as MBC (minimal bactericidal concentration), the lowest concentration of the component at which 100% of bacteria were killed.

Mice

Inbred Balb/c male mice from the breeding unit, Department of Immunology, Jagiellonian University Medical College, Cracow, Poland were used between 6 and 8 weeks of age. The authors were granted permission by the Local Ethics Committee to use mice in this study.

Macrophages

Peritoneal mouse macrophages (Mϕ) were induced by intraperitoneal injection of 1.0 ml of paraffin oil (Sigma, St. Luis, MO, USA). Cells were collected 48 h later by washing out the peritoneal cavity with 5 ml of PBS (phosphate buffer solution) containing 5U heparin/ml (Polfa, Warsaw, Poland). Cells were centrifuged and red blood cells were lysed by osmotic shock using distilled water; osmolarity was restored by addition of 2× concentrated PBS. The presence of macrophages (85–90%) was judged by cytochemical demonstration of non-specific esterase-positive mononuclear cells, using α-naphtyl acetate (Sigma).

Cell culture. Activation of cells for production of inflammatory mediators

Mϕ were cultured in 24-well flat-bottom cell culture plates at 5×10^5/well in RPMI 1640 medium (JR Scientific Inc., Woodland, CA) supplemented with 5% FCS, at 37 °C in an atmosphere of 5% CO_2. Cells were activated with 20 U/ml of IFN-γ (Sigma, Steiham, Germany) and 100 ng/ml of LPS (E. coli 0111 B:4, Sigma Steiham, Germany) and cultured in the presence of test agents. After 24 h culture supernatants were collected and frozen at –80 °C until used.

Measurement of cell viability

Viability of the cells was routinely monitored by cellular exclusion of trypan blue. In some experiments cell respiration, an indicator of cell viability, was assessed by mitochondrial-dependent reduction of MTT to formazan. Cells in 96-well plates were incubated at 37 °C with MTT (0.2 mg ml^{-1} for 60 min). Then, culture medium was removed by aspiration and cells were solubilized in DMSO (200 µl). The extent of reduction of MTT to formazan within cells was quantitated by measurement of absorbance at 550 nm.

Cytokines determination

Cytokine concentrations in culture supernatants were measured using capture ELISA. For IL-6, IL-10, IL-12 determination 96-well plates (Corning, NY, USA) were coated overnight with rat mAb against a mouse cytokine (capture antibody). After blocking the plates with 4% albumin (2 h) or 3% milk standards and tested supernatants were added and incubated overnight. Finally, plates were coated with biotinylated antibodies against the same cytokine detecting antibody for 1h. The ELISA was developed with horseradish peroxidase streptavidin (Vector, Burlingame, CA, USA), followed by *o*-phenylenediamine and H_2O_2 (both Sigma, Steiham, Germany) as substrates 30 min. The reaction was stopped with 3 M H_2SO_4 and the optical density of each well at 492 nm was measured in a plate reader.

IL-6: Rat anti-IL-6 mAbs, clone: MP5-20F3 (2 µg/ml) and biotinylated rat anti-IL-6, mABs clone: MP5-32C11 (0.5 µg/ml) (both Phar Mingen, San Diego, CA, USA) were used as detecting antibodies. Recombinant mouse IL-6 (PeproTech Rocky Hill, New York, USA) was used as a standard. The detection limit was about 15 pg IL-6/ml.

IL-10: Rat anti-mouse IL-10 mAbs, clone: JES5-2A5 (5 µg/ml) and biotinylated rat anti-mouse IL-10 mAbs, clone: JES5-16E3 (2 µg/ml) (PharMingen, San Diego, CA, USA) were used as detecting antibodies. Recombinant mouse IL-10 (Genzyme, Cambridge, UK) was used as a standard. The limit of detection was 30 pg IL-10/ml.

IL-12 p40: Rat anti-mouse IL-12 (p40p70) mAbs, clone C15.6 (2 µg/ml) (PharMingen, San Diego, CA, USA) and biotinylated rat anti-mouse IL-12 mAbs, clone: C17.8 (1 µg/ml) (Endogen, Woburn, MA, USA) were used detecting antibodies. Recombinant mouse IL-12 (Genzyme, Cambridge, UK) was used as a standard. The detection limit was about 30 pg IL-12 p40/ml.

TNF-α: Rat anti-mouse TNF-α mAbs, clone: TN3-19.12 (2 µg/ml) and biotinylated rabit anti-mouse/rat TNF-α pAbs (1.6 µg/ml) (Phar Mingen, San Diego, CA, USA) were used as detecting antibodies. Recombinant mouse TNF-α (Sigma, Steiham, Germany) was used as a standard. The limit of detection was 30 pg TNF-α/ml.

Determination of H_2O_2 production

H_2O_2 detection was based on the oxidation of phenol red by H_2O_2 in the presence of horseradish peroxidase (HRPO) [32]. Briefly, samples were suspended in an assay solution containing 0.56 mM of phenol red and 20 U/ml HRPO (both Sigma, Steiham, Germany) in phenol red-free HBSS medium and seeded in tissue culture plates in a final volume of 100 µl per well. After incubation for 2 h the reaction was stopped by addition of 10 µl of 1N NaOH per well and absorbance was read at 600 nm. Wells with NaOH added at the beginning of testing were used as blanks. H_2O_2 concentration was calculated from H_2O_2 (Sigma, Steiham, Germany) standard curve.

Statistical analysis

If not otherwise stated the statistical significance of differences between groups was analysed using a factorial ANOVA (Microsoft Excel) followed by Student's test, if appropriate. Non-parametric statistical analyses (Mann-Whitney U test) were done using Statistica PL™ v. 6.0 (StatSoft, Poland). Results are expressed as mean ± SE. A p-value less than 0.05 was considered statistically significant.

Results

Stability of TauBr

To determine the stability of TauBr in our experimental setup, UV spectrum of TauBr was analysed. TauCl was used as a reference agent. Time course analysis has shown similar stability of TauBr and TauCl at room temperature for 24 h when incubated alone in a phosphate buffer. At higher temperature (37 °C) the concentration of TauBr decreased by about 20%, while TauCl remained unchanged (Fig. 1).

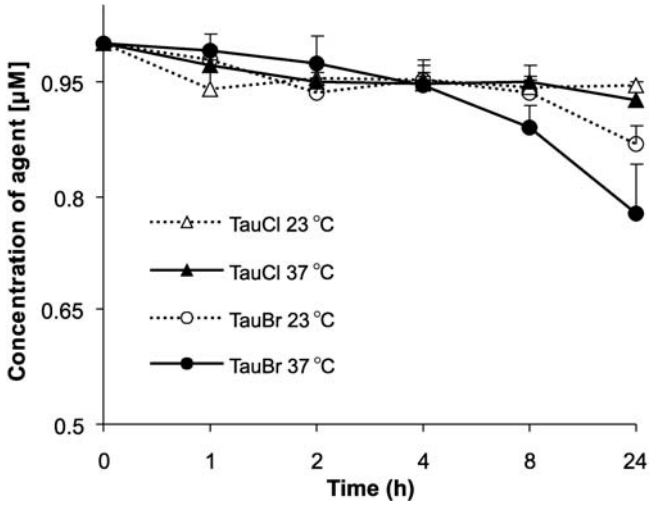

Fig. 1. Stability of TauBr and TauCl in phosphate buffer (pH 7.4) at different temperatures. TauBr and TauCl were monitored by UV absorption spectra and the concentrations of taurine monobromamine and taurine monochloramine were estimated as described in Methods. The results are the means ± SE of 3 separate experiments.

Table 1. Interaction of TauBr with nitrite and hydrogen peroxide.

(A) Nitrite		(B) Hydrogen peroxide	
100 μM of NO_2^- incubated with:	Recovery of NO_2^- (%)	100 μM of H_2O_2 incubated with:	Recovery of H_2O_2 (%)
None (control)	100	None (control)	100
TauBr 1000	84 ± 3	TauBr 1000	0
TauBr 500	94 ± 3	TauBr 500	0
TauBr 250	97 ± 1	TauBr 250	3 ± 1
TauBr 100	**100 ± 1**	**TauBr 100**	**8 ± 3**
TauBr 50	100 ± 1	TauBr 50	58 ± 5**
		TauBr 25	77 ± 3*
HOCl 100	**10 ± 2****	**HOCl 100**	**< 2****
TauCl 100	**95 ± 2**	**TauCl 100**	**99 ± 3**
Tau 1000	101 ± 4	Tau 1000	97 ± 3

The reaction mixture contained: 100 μM NO_2^- (**A**); or 100 μM H_2O_2 (**B**) and TauBr, TauCl, Tau or HOCl at the concentration indicated (μM). Samples were incubated in 0.2 M phosphate buffer (pH 7.4). After 1 h, the level of NO_2^- (**A**) or H_2O_2 (**B**) was measured as described in Methods. The results are the mean ± SE of five separate experiments. *$p < 0.05$; **$p < 0.001$ vs. control.

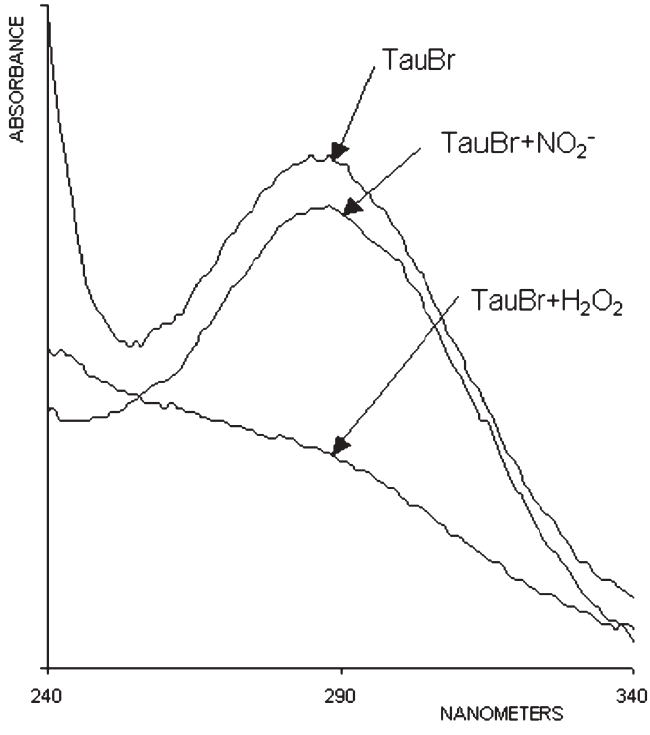

Fig. 2. The UV absorption spectra of TauBr incubated with equimolar concentrations of either NO_2^- or H_2O_2 in 0.2 M phosphate buffer at pH 7.4. Samples of control TauBr were incubated in the buffer alone. After 30 min at 23 °C, absorption spectra were measured against blanks without TauBr.

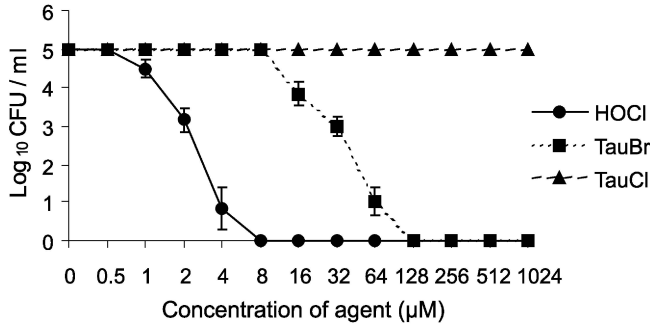

Fig. 3. Bactericidal activity of TauBr, TauCl and HOCl against *E. coli* (EC). Bacteria (1×10^5/ml) were incubated with different concentrations of either TauBr, TauCl or HOCl at phosphate buffer pH.7.4 for 30 min. The reduction of bacteria growth was estimated as described in Methods. Each point on the killing curves represents the mean ± SE calculated from 12 separate experiments.

Interactions of TauBr with hydrogen peroxide (H_2O_2)

Figure 2 indicates that at pH 7.4, TauBr reacts directly with H_2O_2 to produce new compounds with no absorption in the UV spectrum between 210–400 nm. The analysis of H_2O_2 concentrations at the reaction mixture shows the consumption of H_2O_2 by TauBr (Table 1). The reaction is well known: $TauBr + H_2O_2 \rightarrow Tau + H^+ + Br^- + O_2$.

Unlike TauBr, TauCl is not reduced by H_2O_2 [4]. As shown in Table 1, TauCl did not affect the level of H_2O_2. In contrast to TauCl, HOCl consumed H_2O_2 completely when both reagents were used at the equimolar concentrations.

Interactions of TauBr with nitrite (NO_2^-)

Figure 2 shows that at neutral pH, the absorption spectrum of TauBr was not changed by nitrite when both reagents were used at the equimolar concentrations. We also tested a consumption of nitrites by TauBr, HOCl or TauCl by measuring NO_2^- levels in the reaction mixture. As shown in Table 1 (the bolded lines), neither TauBr nor TauCl, when used at equimolar concentrations, decreased the level of NO_2^-. By contrast, nitrites were consumed by HOCl.

Bactericidal activity of TauBr, TauCl and HOCl

TauBr bactericidal activity was tested at pH 7.4, at concentrations ranging from 1 to 1000 μM. In our experimental set-up TauBr exerted strong bactericidal activity to *E. coli* (MBC ~ 100 μM) (Fig. 3). The bactericidal activity of HOCl was significantly higher as compared to TauBr (MBC ~ 8 μM).

Table 2. The role of MPO-halide system products in inflammation.

Agent	Biological activity			
	Immune defense		Host injury	
	Micro-bicidal	Immuno-regulatory	Cytotoxic	[Ref.]
HOCl	(+++)	(–)	(++)	[1, 5, 33]
TauCl	(±)	(+)	(–)	[11, 14, 15]
HOBr	(+++)	**(?)**	(+++)	[7, 10, 22]
TauBr	(++)	(+)	(–)	[4, 25, 26]
H_2O_2	(–)	(+)	(±)	[1, 5, 31]

The hypothetic role of MPO-halide system products at a site of inflammation as suggested by the data from in vitro studies in which MPO-halide system products were tested at physiological concentrations (< 300 µM).

TauCl, in contrast to TauBr and HOCl, at these experimental conditions (< 1000 µM) did not kill *E. coli* bacteria. So, in further experiments bactericidal activity of TauBr was compared only to that of HOCl.

Nitrites and H_2O_2 differentially affect the bactericidal activities of TauBr and HOCl

Under the conditions used, neither nitrite nor hydrogen peroxide exerted bactericidal activity when incubated alone with the test bacteria (data not shown). Interestingly, nitrite and H_2O_2 differentially affected bactericidal activity of TauBr and HOCl.

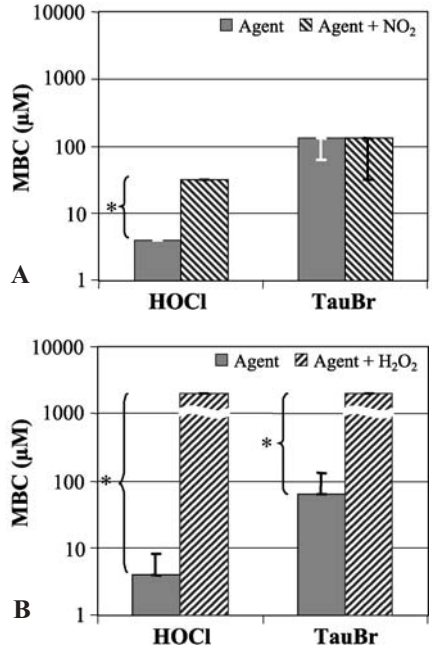

Fig. 4. Influence of nitrite (**A**) and H_2O_2 (**B**) on the bactericidal activity of TauBr and HOCl against EC. TauBr and HOCl were pre-incubated with equimolar concentrations of either NO_2^- or H_2O_2. After 30 min of the incubation at pH 7.4, bacteria (EC) were added (1×10^5/ml) and bactericidal activity of the test samples was estimated as described in the Methods. Nitrites (**A**) and H_2O_2 (**B**) were examined in the separate sets of experiments. The results are shown as MBC values (the concentration that give 100% killing of bacteria) taken from 3 independent experiments for each panel. Mann-Whitney U test was used to test the differences in numerical data between two independent groups. Medians along with interquartile ranges depicted all variables. *p < 0.05

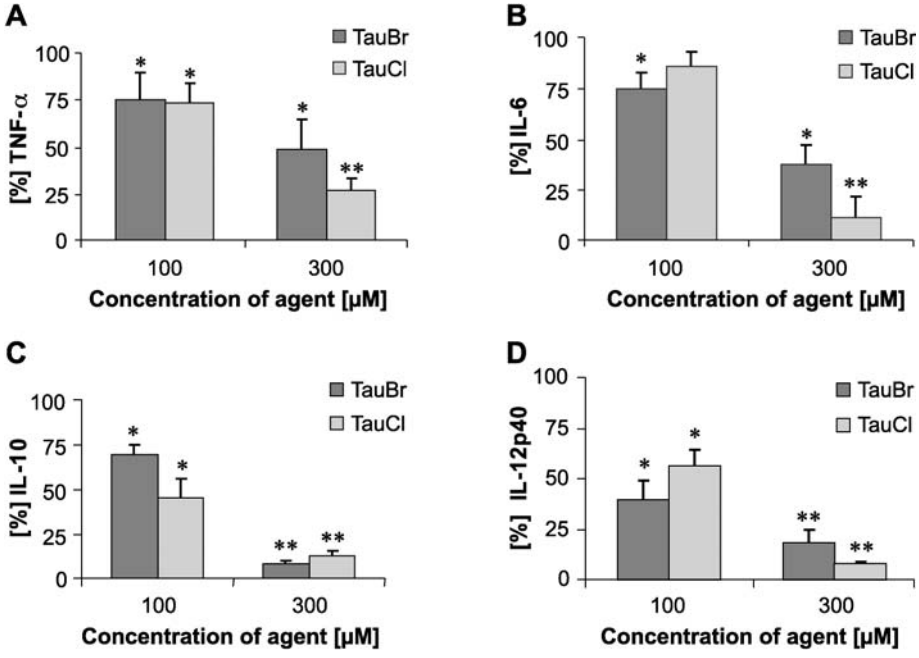

Fig. 5. Effect of TauBr and TauCl on cytokine production by macrophages. Macrophages, stimulated with LPS and IFN-γ were incubated with different concentrations of either TauCl or TauBr. The production of TNF-α (**A**), IL-6 (**B**), IL-10 (**C**) and IL-12p40 (**D**) was estimated as described in Methods. Results are expressed as percentage of the cytokine production by control macrophages. Data were calculated from 11 separate experiments. The production of cytokines by macrophages stimulated with LPS and IFN-γ (control group): TNF-α = 977 ± 449 pg/ml; IL-6 =16.7 ± 5.7 ng/ml; IL-10 = 320 ± 166 pg/ml; IL-12p40 = 7.2 ± 3.7 ng/ml. Taurine alone did not affect the production of cytokines (data not shown). The statistical significance of differences between groups was analysed using a factorial ANOVA. *p < 0.05; **p < 0.01 (Experimental groups vs. control)

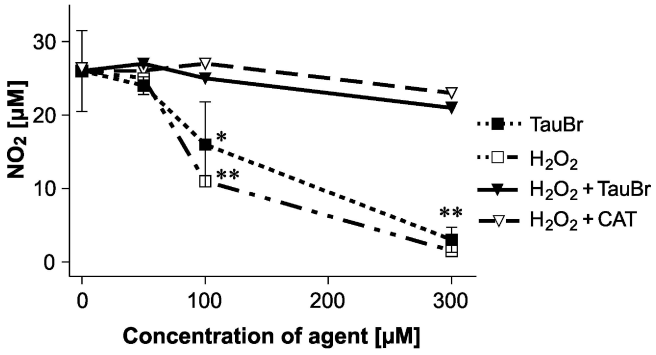

Fig. 6. The effect of H_2O_2 interactions with TauBr on nitrite generation by macrophages. TauBr was pre-incubated with equimolar concentrations of H_2O_2. After 30 min of the incubation at pH 7.4, the test agents, at indicated concentrations, were added to macrophages stimulated with LPS and IFN-γ. After 24 h, supernatants were collected and nitrites were measured using Griess reagents (see Materials and Methods). Results are the mean ± SE of five separate experiments. The statistical significance of differences between groups was analysed using a factorial ANOVA. *$p < 0.05$; **$p < 0.01$ (TauBr vs. TauBr + H_2O_2)

As shown in Fig. 4B, at neutral pH bactericidal activity of both HOCl and TauBr to EC was completely neutralized by H_2O_2 used at equimolar concentrations.

Nitrite also affected bactericidal activity of the test agents, however the effect was different from that evoked by addition of H_2O_2. The bactericidal activity of HOCl was significantly inhibited by nitrite. On the contrary, the effect of nitrite on the bactericidal activity of TauBr was negligible (Fig. 4A).

Effect of TauBr and TauCl on the release of cytokines by stimulated macrophages

To determine TauBr cytotoxic activities against cells involved in inflammation, viability of macrophages incubated with TauBr (1–1000 μM) was tested. In our experimental set-up, TauBr, at concentrations up to 300 μM did not affect viability of macrophages. In further experiments macrophages were cultured with TauBr and TauCl used at non-cytotoxic concentrations (50–300 μM). At these concentrations, HOCl shows cytotoxic effect on macrophages [24].

As shown in Fig. 5, TauBr and TauCl exerted similar, dose dependent, inhibitory effects on cytokine production by peritoneal macrophages stimulated with LPS and IFN-γ. No statistically significant differences were found in the inhibitory activities of 100 μM of TauBr against TNF-α , IL-6, IL-10 and IL-12 p40. At the highest, non-cytotoxic concentration TauBr differentially affected the cytokine production. TauBr showed the strongest inhibitory activity against IL-10, while TNF-α production was inhibited the least.

*Nitrites and H_2O_2 differentially affect the immunoregula*tory *potency of TauBr*

To determine whether NO_2^- and H_2O_2 affect bactericidal and immunoregulatory activities of TauBr in a similar way, the influence of the test agents on generation of selected inflammatory mediators by activated macrophages was studied. As shown in Fig. 6 TauBr and H_2O_2 exerted similar, dose-dependent inhibitory effect on nitrite production by macrophages. Interestingly, the mixture of TauBr and H_2O_2, used at the concentrations in which both agents separately exerted suppressive activity, did not inhibit the production of NO_2^-. TauCl has not been used as a reference agent in these experiments since TauCl does not react with H_2O_2 .

Figure 7 shows the effect of NO_2^- and H_2O_2 interactions with TauBr on IL-10 production. As expected, NO_2^- did not affect the suppressive activity of TauBr on IL-10 release. On the contrary, such suppressive activity of TauBr was completely neutralised by H_2O_2 when these agents were added simultaneously at equimolar concentrations. The same effect was observed when the production of TNF-α, IL-6 and IL-12 was tested (data not shown).

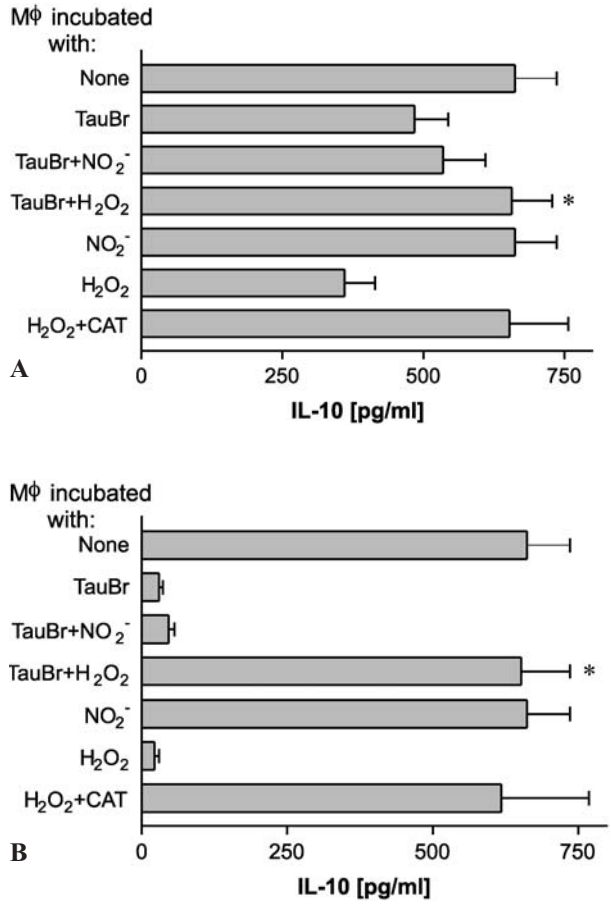

Fig. 7. The effect of nitrite and H_2O_2 interaction with TauBr on IL-10 production by activated macrophages. TauBr was pre-incubated with equimolar concentrations of either NO_2^- or H_2O_2. After 30 min of the incubation at pH 7.4, the test agents, at indicated concentrations (**A** – 100 μM; **B** – 300 μM), were added to the culture of macrophages stimulated with LPS. After 24 h supernatants were collected and the level of IL-10 was measured by ELISA. Results are the mean ± SE of three separate experiments. *TauBr vs TauBr + H_2O_2 **(A)** $p < 0.05$; **(B)** $p < 0.001$

Discussion

Although the biological activities of both chlorinating and brominating oxidants in vitro are in general well characterised, the role of the latter in acute inflammatory response is still the matter of discussion. For years bromide has attracted little attention because its extracellular concentration is at least 1,000-times lower than that of Cl^- [1, 6]. Thus, it could imply that in vivo chlorinating oxidants will strongly compete with brominating oxidants limiting their physiological role to minimum. Meanwhile, it has been reported that neutrophil myeloperoxidase generates chlorinating and brominating oxidants; the production of the latter was previously ascribed solely to eosinophil peroxidase [4]. Moreover, it has been shown that the bromination pathways operate when Cl^- concentrations are 1,000-times to 10,000-times higher than Br^- concentrations [7, 20]. Therefore, the bromination pathways may be physiologically relevant.

In this study we have compared some biological activities of micromolar concentrations of TauBr with these of HOCl and TauCl, the major products of MPO-halide system. These oxidants, generated by monocytes and neutrophils at sites of inflammation, can be involved both in a host defence mechanism and as a means of tissue injury [2, 5, 22]. However, their activity in vivo will depend not only on the local concentrations, but also on their reactions with other reactive species which may result in the formation of products with new biological activities [24, 28, 30]. Indeed, our study confirmed previous reports [4] that at physiological pH (pH 7.4) TauBr, but not TauCl, is decomposed by H_2O_2 to form non active products. On the other hand, nitrite (NO_2^-), a major end-product of nitric oxide metabolism, does not react with TauBr and TauCl. Nitrite, however, competes with taurine for reaction with HOCl to form nitryl chloride (NO_2Cl) [24, 30]. Thus, it is reasonable to speculate that the balance between H_2O_2 and nitrite at sites of inflammation will favour either chlorinating or brominating oxidants (e.g TauCl vs TauBr). The concentration of MPO-halide system products which can be achieved in body fluids is difficult to estimate due to their extremely high reactivity [8]. The chemical basis of these reactions can be reduced to: oxidation of thiols; halide substitution of activated C-H compounds; transhalogation and hydrolytic degradation [5, 12, 23]. This may rise doubts whether in vitro demonstrated activity of chlorinating and brominating oxidants is relevant to the situation in vivo. Nevertheless, in our and others' opinion [8], the physiologically relevant parameter is not the concentration, but rather the amount of HOCl, TauCl or TauBr to which a biological target is exposed. Stimulated neutrophils produce 200 nmol HOCl per 10^7 cells over 30 min [8]. Therefore, it is, possible that the amounts of oxidants used in this study could be achieved in vivo at sites of inflammation. Whether TauBr at the physiological concentrations plays a role in inflammation, is an additional question. Our study has shown that TauBr and HOCl, at similar micromolar concentrations, exert strong microbicidal activity. At these conditions TauCl did not kill bacteria. These results are in agreement with the studies of Gaut et al. [7]. They have shown that addition of low concentration (1 μM) of Br^- markedly increased bactericidal activity of the complete myeloperoxidase-H_2O_2–Cl^- system in vitro. Therefore, one may suggest that physiologically plausible variations in Br^- concentration will support neutrophil dependent defence system (HOCl/TauCl) by formation of HOBr and TauBr, very strong bactericidal agents. Importantly, TauBr, in contrast to HOCl, at bactericidal concentrations (< 200 μM) does not exert cytotoxic activity. The contribution of chlorinating and brominating oxidants in bacterial killing, in the regulation of inflammatory cells function and in tissue injury will also depend on the interactions with other biologically active agents present at a site of inflammation. For example, activity of HOCl may be neutralised by both, nitrites and H_2O_2 [28, 33], while activity of TauBr is affected only by the presence of H_2O_2 [4, 7].

In the present study we have also examined immunoregulatory potential of TauBr. The ability of TauBr to suppress the production of inflammatory mediators by activated macrophages was compared with that of TauCl. Both taurine haloamines inhibited the generation of nitrites and cytokines (TNF-, IL-6, IL-12, IL-10) in a similar, dose-dependent manner. These results are in agreement with our recent findings [26]. We have shown that TauBr and TauCl can induce expression of heme oxygenase-1 (HO-1), a stress inducible protein, in both non-activated and LPS-activated macrophages. Importantly, dose-dependent induction of HO-1 was associated with a concomitant fall in NOS-2 protein level. Thus, at a site of inflammation, TauCl and TauBr may provide a link between taurine-dependent and HO-1-dependent cytoprotective mechanisms.

In conclusion, the present study, together with previous reports, suggests that TauBr may be a part of neutrophil defence system [7]. TauBr is generated by neutrophil MPO-halide system and at low non-toxic concentrations exerts in vitro bactericidal activity, even stronger than TauCl. Now, TauBr contribution to pathogen killing by phagocytes in vivo remains to be established. TauBr shows similar to TauCl capacity to modulate inflammation by induction of heme oxygenase expression and by inhibition of inflammatory mediators generation by activated macrophages. Thus, bromide may be a previously unexpected, but important component of neutrophil activity at a site of inflammation. On the other hand, TauBr may be easily neutralised by hydrogen peroxide generated by both bacteria and phagocytes at a site of inflammation. Nevertheless, further studies are necessary to compare the effect of TauBr and TauCl on the balance between pro-(TNF-α) and anti-(IL-10) inflammatory cytokines production by the cells engaged in inflammation.

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